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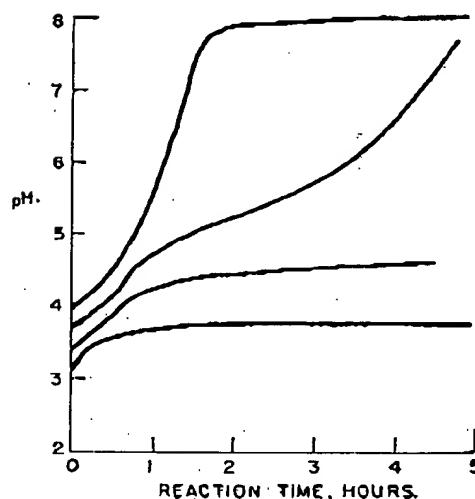


Fig. 3.—Change of pH as a function of initial acidity during oxidation of 80% dialdehyde starch.

various initial pH values (obtained by varying the amounts of acetic acid) but with other conditions held constant. The chlorous acid oxidation (Equation 1) results in consumption

of protons and, consequently, a minimum level of total acidity must be present for complete reaction. Our results indicate that this requirement is met when the mole ratio chlorite/acetic acid is at least 2.

Discussion

Chlorous acid has proved to be an excellent oxidizing agent for the quantitative conversion of dialdehyde starches to the corresponding dicarboxyl starches. Analyses for carbonyl and carboxyl reveal the highly specific action of this oxidant which minimizes degradation of the dialdehyde starch. Retention of the granule form at dicarboxyl contents of 20% and below as well as the ability to form highly viscous aqueous dispersions is evidence that slight, if any, molecular degradation occurs during the oxidation. The solution properties as well as other physical and chemical characterizations of these materials are now under investigation and will be described separately.

Acknowledgment.—The authors wish to acknowledge the assistance of Mr. Darrell D. Ebbing in many of the preparations and analyses required and of Mrs. Clara E. McGrew for the sodium analyses.

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[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, PURDUE UNIVERSITY]

Oxidation of Amylopectin with Hypochlorite at Different Hydrogen Ion Concentrations^{1,2}

BY ROY L. WHISTLER AND RICHARD SCHWEIGER

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The principal organic products from oxidation of corn starch amylopectin with hypochlorite at different pH values are glyoxylic and D-erythronic acids. These products are obtained in maximum yield at a pH slightly above 7. Reaction is envisioned as formation of a carbonyl on carbon C₁ or C₆, enediol formation involving C₁ and C₆, addition of hypochlorous acid and subsequent further oxidation to cleave the carbon-carbon bond and produce a dicarboxylated unit in the amylopectin chain.

Oxidative reactions are of importance to industries processing polysaccharides but are of greatest importance to those industries producing starch and cellulose. Starch is often purposefully oxidized to give it new and useful properties while cellulose is unintentionally oxidized when pulps and cellulosic textiles are bleached. The most common oxidant, used in these instances, is sodium hypochlorite. Although chlorine is often used in bleaching cellulose it is commonly added to an alkaline system where it is transformed to hypochlorite. As a consequence, some importance is attached to the establishment of the mechanism through which hypochlorite reacts with polysaccharides. One of the first steps must be the determination of the nature of the oxidation products formed. Earlier, it has been shown that hypochlorite at pH 9 and 11 reacts with corn amylose and with methyl 4-O-methyl-D-glucopyranosides at 25° to produce ex-

tensive cleavage of anhydro D-glucose units between carbon atoms 2 and 3. This evidence is based on isolation of D-erythronic acid, its γ -lactone, glyoxal and glyoxylic acid.

The present work provides information concerning the effect of pH on the oxidation rate and on the type and amount of oxidation products when hypochlorite reacts with amylopectin. Corn amylopectin is used in place of corn amylose to avoid retrogradative effects. Although amylopectin molecules are branched, the degree of branching is not great and most of the glycosidic linkages are of the α -D-(1 → 4) type which almost exclusively form the glycosidic linkages of amylose. It is not expected, therefore, that the mechanism of amylopectin oxidation should be greatly different from that of amylose.

The rate at which hypochlorite oxidizes amylopectin in solution is greatly influenced by hydrogen ion concentration. When amylopectin at 1.25% concentration in water is treated with sodium hypochlorite at a level of two moles of oxidant per mole of anhydro D-glucose unit, the oxidant is consumed at a rate indicated in Fig. 1. This graph, or a plot of the period of time for half consumption of

(1) Paper presented before the joint Meeting of the Division of Carbohydrate Chemistry and Division of Cellulose Chemistry at the 131st Meeting of the American Chemical Society at Miami, Florida, April, 1957.

(2) Journal Paper No. 1160 of the Purdue Agricultural Experiment Station.

(3) R. L. Whistler, E. G. Linke and S. Kuzniac, THIS JOURNAL, 78, 4704 (1956).

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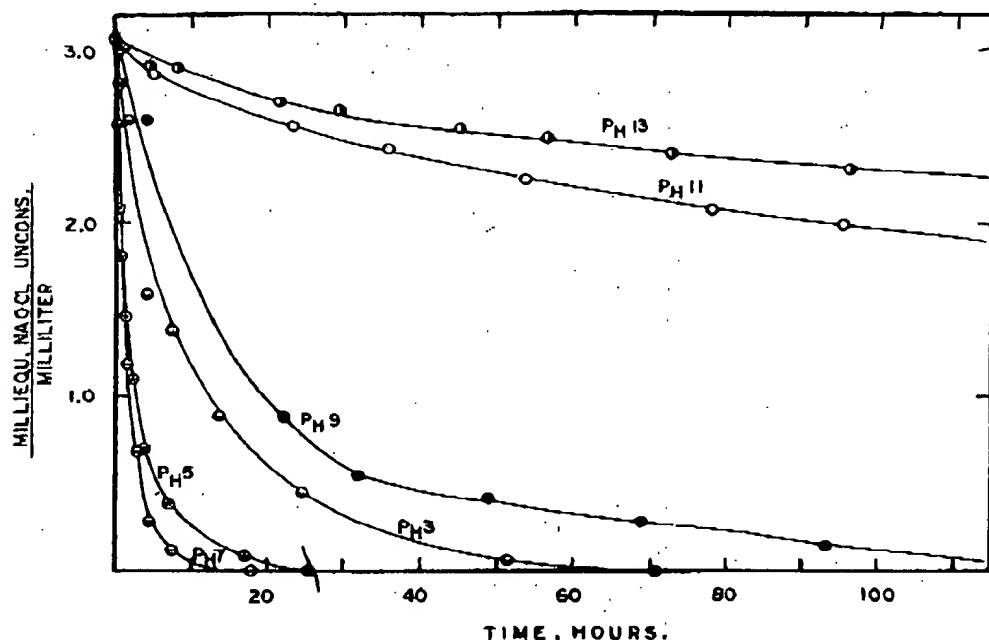


Fig. 1.—Hypochlorite consumption by starch amylopectin at 25°.

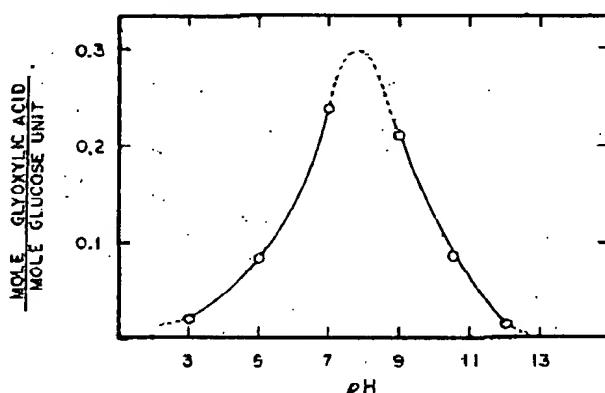
the hypochlorite against ρH , illustrates that the reaction is most rapid at ρH 7. However, at ρH 7 and regardless of the amount of oxidant present within the range examined (0.5 to 3 mole of hypochlorite per mole of anhydro D -glucose unit), 29% is converted to chlorate. At other ρH values much less chlorate formation occurs as is shown in Table I.

TABLE I

ρH	8	5	7	9	10.5	12
Hypochlorite to chlorate, %	1.5	7.1	28.8	4.6	0.7	0.5

For industrial modification of starch with hypochlorite, the ρH selected is usually 9 or 11. In these alkaline conditions oxidation proceeds slowly. At ρH 10.5 more than a month is required for the consumption of 3 moles of hypochlorite per mole of anhydro D -glucose unit while at ρH 13, oxidant still remains after a reaction period of 8 months.

Regardless of ρH level the same principal organic oxidation products are found on hydrolysis of the oxidized amylopectin. These products are glyoxylic and D -erythronic acids as previously shown⁸ for ρH 9 and 11. Quantitative measurement of glyoxylic acid shows maximum production at near ρH 7 (see Fig. 2). These data would suggest that hypochlorite cleaves the bond between carbons C2 and C3 at all ρH levels but is most specific at near ρH 7. Here about 25% of the oxidant not converted to chlorate is consumed in bond cleavage. Hypochlorite not converted to chlorate nor consumed in the C2-C3 bond cleavage presumably acts to bring about random splitting of the polysaccharide with production of a large quantity of carbon dioxide (Table II), a diversity of organic products but few of low molecular weight. Evidence for lack of low molecular weight organic com-

Fig. 2.—Amount of glyoxylic acid produced at different ρH values by action of 3 moles of hypochlorite per D -glucose unit of amylopectin (values corrected for hypochlorite converted to chlorate).

ponents is based on analysis of the dialyzate obtained from the oxidized amylopectin. Concentration of the dialyzate and subsequent chromatography fail to show low molecular weight frag-

TABLE II

ρH	3	5	7	9
Moles of carbon dioxide per mole D -glucose unit per 8 moles of hypochlorite consumed as oxidant	0.84	0.69	0.58	0.59

ments such as glyoxal or glycolic, oxalic and D -glucuronic acids. Evidence of some non-mobile component at the chromatographic starting line might suggest the presence of moderately high molecular weight components. When the dialyzate is hydrolyzed and chromatographed evidence is obtained for the presence of glyoxylic acid, D -erythronic acid and D -glucose.

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Chlorate formed in the reactions is not an oxidant for amylopectin nor for hypochlorite oxidized amylopectin at the pH values examined of 3, 5 and 7. Thus after addition of chlorate to solutions of amylopectin or hypochlorite oxidized amylopectin, at these pH values, the total oxidant can be found after several days.

At all pH values and even at hypochlorite levels equivalent to three moles per mole of anhydro D-glucose unit some sugar units remain unattacked. Thus, in each instance, hydrolysis of the hypochlorite oxidized amylopectin permits the isolation of D-glucose identified in its crystalline form and as its phenylosazone.

In acid solution, hypochlorite also oxidizes the primary alcohol group as evidenced by the chromatographic appearance of D-glucuronic acid in the hydrolysis products. Only small amounts of D-glucuronic acid appear to be produced at pH 5 but more is evident at pH 3. If chlorine is introduced at pH 3 for several hours over a 5-day period, large amounts of D-glucuronic acid are produced. Formation of uronic acids by hypochlorous acid and acidic solutions of chlorine are described by others.^{4,5} Oxidative reactions under these low pH conditions must be random since no single organic molecular species is predominant in the reaction products.

Under strongly alkaline conditions the oxidation is slow and is subject to the interference of alkaline degradations.⁶ Such interference should become more marked the higher the alkalinity but should be significant in all alkaline solutions. Alkaline degradation or modification of either intermediates or products probably accounts for the lower reducing power of hydrolyzates which are obtained from amylopectin oxidized at high pH values (Fig. 3).

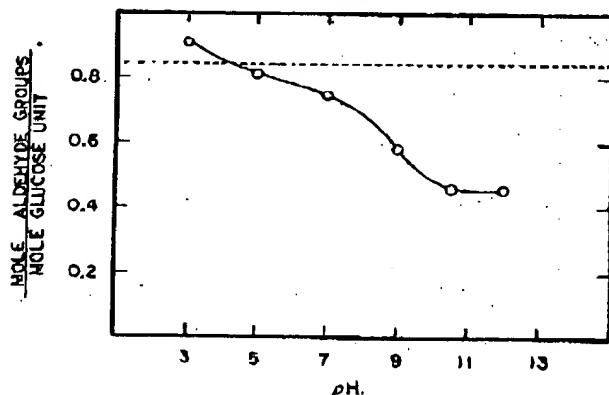


Fig. 3.—Variation in aldehyde content of hydrolyzed product from oxidation at different pH values. Broken line represents aldehyde groups measured in hydrolyzed but un-oxidized amylopectin.

Hypochlorite oxidation of amylopectin becomes most specific between the pH values of 7 and 9 with

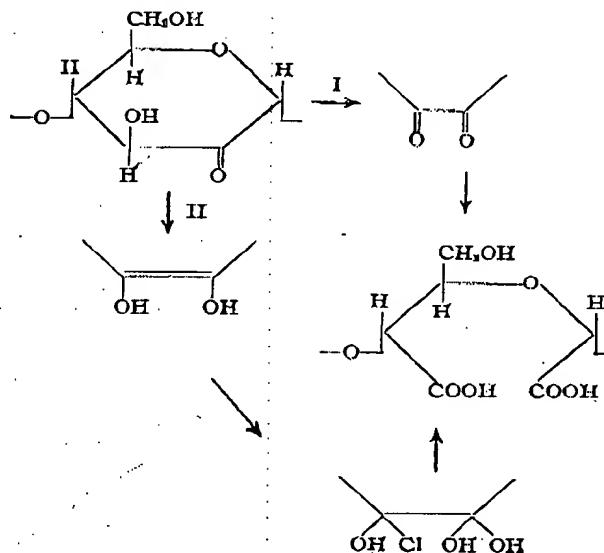
(4) M. E. McKillop and C. B. Purves, *Can. J. Chem.*, **32**, 312 (1954).

(5) D. H. Grangford, E. K. Gladding and C. B. Purves, *Paper Trade J.*, **116**, No. 7, 41 (1942).

(6) W. M. Corbett and J. Kounor, *J. Chem. Soc.*, 1431 (1955); R. L. Whistler and J. N. BeMiller, *Advances in Carbohydrate Chem.*, in press.

the maximum specificity near pH 7. In this region the major organic products isolated after hydrolysis are glyoxylic and D-erythronic acids. These and further organic products are formed in smaller amounts at other hydrogen ion concentrations. Maximum rate of the oxidation also occurs at or near pH 7.

The oxidative reaction may be envisioned as following one of several courses but the two most likely are illustrated



The first step must involve oxidation of the secondary hydroxyl group on either carbon atoms C2 or C3 with the formation of a carbonyl group. Such oxidation probably proceeds through the hypochlorite ester as an intermediate. Continued oxidation might involve the adjacent secondary hydroxyl group to eventuate in an α,α -dicarbonyl which readily would oxidize further with cleavage of the carbon-carbon bond to give two carboxyl groups as shown in scheme I.

On the other hand, following introduction of one carbonyl group an enediol could develop and subsequently add hypochlorous acid to produce an intermediate that will further oxidize rapidly to the dicarboxyl end-product (scheme II).

Reaction according to scheme II seems to be most probable from an examination of the conditions which favor most rapid reaction. Hypochlorous acid may be assumed the active oxidant which initially attacks the secondary alcohol group because the oxidation proceeds most swiftly in pH ranges where hypochlorous acid is abundant.⁷ Attack upon the secondary hydroxyl does not seem to be the rate-determining step, however, since the maximum rate of oxidation is found somewhat above pH 7, whereas undissociated hypochlorous acid is present in maximum concentration at pH 5. Under the more alkaline conditions enolization of the initial oxidation product would be favored with production of the enediol of scheme II. It might be expected that at pH values slightly above 7 enolization proceeds sufficiently rapidly to favor

(7) O. Holst, *Chem. Revs.*, **55**, 169 (1954).

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the next phase which would be addition of hypochlorous acid to the double bond and subsequent oxidation of this product to produce a dicarboxylic acid. Polysaccharide units thus converted to the dicarboxyl derivative would yield on hydrolysis equal molar quantities of glyoxylic and D-erythronic acids.

Experimental

Amylopectin.—Commercial corn starch was fractionated by the pentasol method of Wilson, Schoch and Hudson.⁸ Amylopectin was obtained from the concentrated centrifugate by precipitation into ethanol and dried by four successive passages through fresh absolute ethanol. During each filtration care was taken not to draw air through the filter cake. Ethanol was removed from the final cake by placing it in a vacuum desiccator over calcium chloride. The product was a fine white powder.

Oxidation of Amylopectin.—Thirty grams of amylopectin was dissolved in 1.5 l. of boiling water and the solution stirred for 2 hr. The solution was then cooled to room temperature, filtered through a linen cloth, made up to a volume of 1.8 l. and divided into six equal parts. To each part was added 56.8 ml. of solution 2.2 N in sodium hypochlorite to provide 2 moles of hypochlorite per mole of D-glucose unit. The volume of each was made to 400 ml. and adjusted to pH values of 3, 5, 7, 9, 11 and 13 by appropriate addition of either hydrochloric acid, sodium bicarbonate, sodium carbonate or sodium hydroxide as required. Solutions were maintained at 25° in the dark and pH values were continuously checked and corrected where necessary. For solutions maintained at pH 5 a small quantity of sodium dihydrogen phosphate was added to buffer the reaction. Hypochlorite consumption was followed by sodium thiosulfate titration of acidified aliquots containing excess potassium iodide. Optical rotation decreased in each instance from about $[\alpha]^{25}\text{D} +2.1^\circ$ to about $+1.7^\circ$. The rate of hypochlorite consumption is shown in Fig. 1. Conversion to chlorate is quite small except at pH 7 where about 29% of the hypochlorite is converted. The actual percentages of hypochlorite transformed at different pH values to chlorate are given in Table I. At pH 7, the percentage conversion of hypochlorite to chlorate remains constant throughout the entire reaction. Also at pH 7, 8 moles of oxidant per sugar unit are consumed in about the same period as required for 2 moles per sugar unit.

Hydrolysis of the Oxidized Amylopectin.—Samples from the above oxidations were each dialyzed against distilled water for 4 days. The dialyzate was concentrated to small volume and a portion chromatographed on paper with butanol:acetic acid:water (4:1:5 v/v) as irrigant. No evidence of small molecules or of mono- or oligosaccharides was observed, although a short streak from the starting point was observed.

The non-dialyzable portions were separately hydrolyzed in 1 N sulfuric acid at 100° for 5 hr. Sulfate was removed as the barium salt after partial neutralization with barium hydroxide. Acidity was maintained by addition of a small amount of hydrochloric acid to prevent precipitation of barium salts of organic acids. The concentrated hydrolyzates were chromatographed on paper with ethyl acetate:acetic acid:formic acid:water (18:3:1:4 v/v). Triplicate papers were developed with ammoniacal silver nitrate,⁹ aniline phthalate¹⁰ and hydroxylamine-ferric chloride, respectively.¹¹ In all samples evidence was found for D-glucose, glyoxylic acid, D-erythronic acid and D-erythronolactone, but not for other products. Glyoxylic acid and D-erythronic acid and its lactone were observed in only trace amounts from amylopectin oxidized at pH 8. Only slightly higher amounts of these substances were observed at pH 5. However, at pH 7 and 9 these compounds appeared to be present in abundance. During hydrolysis of samples oxidized at pH 8 and 5, the solution became dark and a brown flocculent precipitate formed which might be suggestive of decomposition of ketosugars.

To determine whether the type of product varied with the

(8) E. J. Wilson, Jr., T. J. Schoch and C. S. Hudson, *This Journal*, 65, 1380 (1943).

(9) S. M. Partridge, *Nature*, 168, 270 (1946).

(10) S. M. Partridge, *Ibid.*, 164, 448 (1949).

(11) M. Abdel-Akher and F. Smith, *This Journal*, 73, 5859 (1951).

level of hypochlorite, amylopectin solutions were oxidized at hypochlorite levels of 0.5, 1.0, 2.0 and 3.0 moles per mole of sugar unit and at pH levels of 7, 9 and 11. When all hypochlorite was consumed the samples were dialyzed against distilled water and hydrolyzed as described above. Paper chromatographic analysis of the hydrolyzed dialyzate showed only the products found above. The principal difference was an expected decrease in D-glucose and an increase in glyoxylic acid with increased levels of hypochlorite. These estimates were obtained by weighing the paper occupied by particular components when mixtures had been applied in a quantitative manner.

Isolation of Glyoxylic and D-Erythronic Acid.—Five-gram portions of amylopectin were oxidized at pH 7 and 9 with hypochlorite at levels of 2 moles per mole of sugar unit and were dialyzed and hydrolyzed as indicated above. The hydrolyzates were concentrated to 70 ml. and extracted with diethyl ether for 6 days. At the end of this time only D-glucose remained in the aqueous phase as indicated by paper chromatography. The ether extract which contained glyoxylic and D-erythronic acid was divided into two parts.

To the first part, after evaporation of ether, was added 10 ml. of water and 10 ml. of an 0.5% solution of 2,4-dinitrophenylhydrazine in 2 N hydrochloric acid. After heating on steam-bath for 1 hour the yellow precipitate was filtered and dried; m.p. 184–189°. On recrystallization from an ethanol-water mixture the melting point was 185–187° and was unchanged by admixture with authentic glyoxylic 2,4-dinitrophenylhydrazone.

The second part, after evaporation of ether, was dissolved in a hot mixture of ethyl acetate and *n*-amyl acetate (3:1). On cooling and partial evaporation of solvent, long prismatic crystals separated, m.p. 104°, unchanged on admixture with authentic D-erythronolactone, $[\alpha]^{25}\text{D} -68.0 \pm 2.5^\circ$ (c 0.6 in water).

Identification of D-Glucose.—The diethyl ether-extracted hydrolyzate described in the preceding section was treated with phenylhydrazine in acetic acid and heated on the steam-bath. The precipitate which formed was filtered, washed with ethanol and dried; m.p. 202–203°. On recrystallization from 70% ethanol it melted at 212° and was undepressed when mixed with authentic D-glucose phenylszone.

Another portion of the ether extracted hydrolyzate was treated with charcoal, concentrated to a sirup and triturated with ethanol. D-Glucose crystallized, m.p. 144–146°, undepressed with authentic material, $[\alpha]^{25}\text{D} +52.5$ (c 1.0 in water, equilibrium).

Determination of Amounts of Glyoxylic Acid Found at Different pH Values.—Amylopectin was dissolved as described above to produce a solution of 1.2% final concentration. Prior to final volume adjustment, the solution was divided into 6 parts and the pH values adjusted to form a series with values of 3, 5, 7, 9, 10.5 and 12. To each was added 2.2 N sodium hypochlorite solution to provide 3 moles of oxidant per mole of D-glucose unit. Solutions were maintained at 25° and pH values continuously adjusted as needed. After all oxidant was consumed 2-ml. aliquots were removed for determination of amount of chlorate present. The solution at pH 12 still contained 23% of its hypochlorite at the end of 3 months and was analyzed at this point. Each solution was neutralized with sulfuric acid and then acidified with 8 ml. of concentrated acid. Sulfur dioxide was then passed through each solution for 20 minutes to reduce chlorate and the material was hydrolyzed at reflux for 6 hr. Sulfate was removed by addition of barium hydroxide. The solution was kept slightly acidic with hydrochloric acid, cooled slowly to room temperature, filtered and concentrated *in vacuo* to 100 ml. Then 20 ml. of each were neutralized with N sodium hydroxide and aldehyde groups determined by the method of Willstätter and Schudel.¹² Equivalent results were obtained by conducting the oxidation in a solution buffered to pH 9 (Fig. 3). Both D-glucose and glyoxylic acid are oxidized. Oxalic acid formed was precipitated with calcium acetate from a solution buffered with sodium acetate. Calcium oxalate removed by filtration was determined by permanganate oxidation. Results are shown in Fig. 2.

To determine whether the chlorate formed in the reaction also oxidized the amylopectin, the effect of this oxidant was ex-

(12) R. Willstätter and O. Schudel, *Ber.*, 61, 780 (1928).

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amined. Amylopectin in 1.25% solutions was treated with six equivalents of potassium chlorate at pH 3, 5 and 7. No oxidant was consumed in 5 days at room temperature. Even amylopectin oxidized by hypochlorite at pH 7 was not oxidized by chlorate when the pH was reduced to 8.

Carbon Dioxide Evolved.—Carbon dioxide produced during oxidation of amylopectin with hypochlorite was determined for reactions at pH 3, 5, 7 and 9. For each determination 5 g. of amylopectin was dissolved in 200 ml. of hot water, 92.5 ml. of 2 N sodium hypochlorite solution (8 moles/mole D-glucose unit) was added and the solution was made up to 450 ml. Concentrated sodium hydroxide solution or hydrochloric acid was added as necessary to produce the desired pH values. After the oxidant was consumed each solution was acidified with about 4 ml. of concentrated sulfuric acid in 20 ml. of water and the mixture refluxed in a contained system swept by a slow stream of nitrogen. Carbon dioxide evolved was measured by passage into a 0.5 N solution of sodium hydroxide. Differential titration of the

alkali with 0.1 N oxalic acid, after addition of barium chloride solution in excess of the carbonate present, gave a measure of the carbon dioxide absorbed.

Since in oxidations at pH 3 and 5 the produced carbon dioxide is immediately evolved, the oxidation was performed in a flask swept by nitrogen. The gas stream was passed into sodium hydroxide as before, but chlorine as well as carbon dioxide was absorbed. Thus the amount of alkali neutralized by chlorine was also determined by treating the solution, titrated to the neutral point, with iodide and concentrated hydrochloric acid and titrating of iodine with thiosulfate. Carbon dioxide produced by the oxidation at pH 3 and 5 is shown in Table II.

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LAFAYETTE, INDIANA

[CONTRIBUTION NO. 28 FROM THE OLYMPIC RESEARCH DIVISION, RAYONIER INC.]

Graded Acid Hydrolysis Studies of a Xylan Polyuronide Associated with Wood Cellulose from Western Hemlock¹

BY J. KELVIN HAMILTON AND NORMAN S. THOMPSON

RECEIVED JULY 8, 1957

A series of oligosaccharides and oligouronides has been obtained by the graded acid hydrolysis of a xylan polyuronide associated with a wood cellulose from western hemlock. At least six distinct acidic substances composed of 4-O-methyl-D-glucuronic acid and D-xylose and four reducing oligosaccharides composed of D-xylose were detected by suitable chromatographic techniques. Three of the oligosaccharides showed the same mobility as authentic specimens of xylobiose, xylotriose and xylotetraose on all chromatographic solvents that were tried. The acidic substances were 4-O-methyl-D-glucuronic acid, 2-O-(4-O-methyl- α -D-glucuronopyranosyl)-D-xylose and an aldotriouronic acid which crystallized as a trihydrate. This acid has been identified by methylation studies as O- α -4-O-methyl-D-glucuronopyranosyl-(1 \rightarrow 2)-O- β -D-xylopyranosyl-(1 \rightarrow 4)-D-xylopyranose. A tentative structure of the parent xylan polyuronide based upon the oligosaccharides and oligouronides that have been identified is described.

The hemicelluloses which remain associated with wood cellulose produced from western hemlock by the sulfite process have been shown to be a mixture of two families of polysaccharides, namely, glucomannans and xylan polyuronides.² The predominant polysaccharides were found to be the glucomannans in which the ratio of D-glucose to D-mannose was 1 to 3.³ The xylan polyuronides varied in their ratio of 4-O-methyl-D-glucuronic acid to D-xylose from 1 to 4 to 1 to 8. Controlled periodate oxidation studies were carried out on these two types of hemicelluloses. These data, in conjunction with specific rotations of the polysaccharides before and after hydrolysis, indicated that both of the above hemicellulose series were linked predominantly by 1 \rightarrow 4- β -glycosidic bonds. Prolonged periodate oxidation, followed by reduction and hydrolysis, also indicated that the glucomannans were predominantly straight chain and the xylan polyuronides were slightly branched. Methylation and graded hydrolysis studies on the glucomannans are in agreement with these results.⁴ Recent studies have shown that the xylan polyuronides can be easily separated from the glucomannans by suitable extraction procedures.⁴ The xylan polyuronides used in these experiments were isolated by a modifi-

cation of one of these procedures (see Experimental).

Information concerning the sequence of the sugar residues in these xylose-containing hemicelluloses may be obtained by stepwise degradation and characterization of oligosaccharides or oligouronides of varying degree of polymerization. This paper is concerned with the partial degradation of the xylan polyuronide components of this wood cellulose system by graded acid hydrolysis and the isolation, identification and characterization of certain members of the oligouronides so obtained.

In many instances acid-containing polysaccharides have been hydrolyzed and found to give large amounts of aldotriouronic and aldotriouronic acids. In only a few instances has the monomeric uronic acid been isolated in good yields under normal conditions of hydrolysis.⁵ The studies of Haworth,⁶ Anderson⁷ and O'Dwyer⁸ on plant gums and hemicelluloses from various plant sources are classics in this field. Anderson and Otis⁹ in 1930 reported the isolation of a methylhexuronic acid from mesquite gum. Later, Anderson¹⁰ and Sands^{11,12}

(6) E. Anderson, M. G. Seeley, W. T. Stewart, J. C. Redd and D. Westerbeck, *J. Biol. Chem.*, **188**, 189 (1940).

(7) W. N. Haworth and E. G. V. Percival, *J. Chem. Soc.*, 2850 (1931).

(8) E. Anderson and S. Elmsman, *J. Biol. Chem.*, **96**, 30 (1931).

(9) Miss M. H. O'Dwyer, *Biochem. J.*, **30**, 658 (1939).

(10) E. Anderson and D. L. Otis, *THIS JOURNAL*, **58**, 4461 (1930).

(11) E. Anderson, J. Kesselman and E. C. Bennett, *J. Biol. Chem.*, **160**, 563 (1941).

(12) Miss L. Sands and W. Y. Gary, *ibid.*, **101**, 578 (1938).

(13) Miss L. Sands and P. Nutter, *ibid.*, **110**, 17 (1939).

(1) Presented at Symposium on "The Chemistry of Lignin, Polysaccharides and Related Substances," Tucson, Ariz., September, 1956.

(2) J. K. Hamilton, H. W. Kircher and N. S. Thompson, *This Journal*, **78**, 2508 (1956).

(3) J. K. Hamilton and H. W. Kircher, presented at the 132nd meeting of the A.C.S., New York, N. Y., September, 1957.

(4) J. K. Hamilton and G. R. Quimby, *Tappi* **60**, 781 (1957).

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